Application of a modified denitrifying bacteria method for analyzing groundwater and vadose zone pore water nitrate at the Hanford Site, WA, USA.

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The Hanford Site in southern Washington contains a large proportion of the 250,000 metric tons of nitrate estimated to reside at DOE sites across the USA. Nitrate concentrations >600 mg/L have been reported for Hanford groundwaters, where nitrate commonly accompanies elevated levels of radionuclide contamination. Much of the Hanford nitrate is stored in the vadose zone, where complicated hydrology and poorly understood chemical and biochemical processes lead to uncertain fate and transport. Analysis of the nitrogen and oxygen isotopic composition of nitrate provides a promising method to identify sources and investigate biochemical degradation of nitrate in the subsurface at Hanford. A preliminary investigation of NO_{$_3$-} fate and transport at the Hanford Site focuses on pore water nitrate, extracted by 1:1 sediment to DI water rinses of vadose zone sediments, in a vertical profile through a radionuclide plume at the TX-TY tank farm, and compares these results with transects across major nitrate plumes in the Hanford unconfined aquifer.

Until recently, methods for analyzing d^{^{15}}N and d^{^{18}}O of NO_{$_3$-} in waters were unwieldy for routine analyses of dilute groundwaters and pore water extracts from vadose

zone sediments; however, Sigman, *et al*., 2001 (Anal. Chem) and Casciotti, *et al*., 2002 (Anal. Chem), developed a method using denitrifying bacteria with a truncated enzymatic pathway to generate N_{$_2$}O for analyzing both isotopes simultaneously from NO_{$_3$-} in dilute samples. Our modifications to this method decrease both culture preparation and sample processing times. Culturing time has been reduced to 2-3 days by increasing the initial inoculation volume to 2mL in 100mL. We grow cultures on a bench top and mix by inversion twice daily, instead of growing on a constant shaking unit. These changes have not been shown to affect the cell yield nor the N_{$_2$}O levels in blanks. Secondly, cell preparation for NO_{$_3$-} reduction has been modified to decrease sample processing time. Intense centrifugation was found to be unnecessary and loose pellet formation at a lower *g* is more than adequate. We found no detectable background level of N_{$_2$}O in the cultures, and have reduced the sample venting time. Lastly, we have found that samples can be injected, cells lysed, and N_{$_2$}O run in the same day with negligible affects to N_{$_2$}O yield and d^{^{15}}N/d^{^{18}}O values.